

11-2015

Detection of genetic and epigenetic DNA markers in urine for the early detection of primary and recurrent hepatocellular carcinoma

Hie-Won Hann

Thomas Jefferson University Hospital, Hie-Won.Hann@jefferson.edu

Surbhi Jain

JBS Science Inc.

Ting-Tsung Chang

National Cheng Kung University Medical Center

Chi-Tan Hu

Buddist Tzu Chi General Hospital and Tzu Chi University

Selena Lin

*Drexel University, College of Medicine**See next page for additional authors*Follow this and additional works at: <http://jdc.jefferson.edu/gastrohepposters>Part of the [Gastroenterology Commons](#), and the [Hepatology Commons](#)

Recommended Citation

Hann, Hie-Won; Jain, Surbhi; Chang, Ting-Tsung; Hu, Chi-Tan; Lin, Selena; Song, Wei; and Su, Ying-Hsiu, "Detection of genetic and epigenetic DNA markers in urine for the early detection of primary and recurrent hepatocellular carcinoma" (2015). *Department of Gastroenterology and Hepatology Posters*.

<http://jdc.jefferson.edu/gastrohepposters/1>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Gastroenterology and Hepatology Posters by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Hie-Won Hann, Surbhi Jain, Ting-Tsung Chang, Chi-Tan Hu, Selena Lin, Wei Song, and Ying-Hsiu Su

Introduction

Hepatocellular carcinoma (HCC) or liver cancer is an aggressive disease and one of the fastest growing cancers by incidence in the United States. Early detection is the key for effective treatment of HCC as the 5-year survival rate is 26% in early stage HCC as compared to only 2% when found after spreading to distant organs. The current marker, alpha-feto protein (AFP) and its fucosylated glycoform, L3, are of limited value with only 40-60% sensitivity.

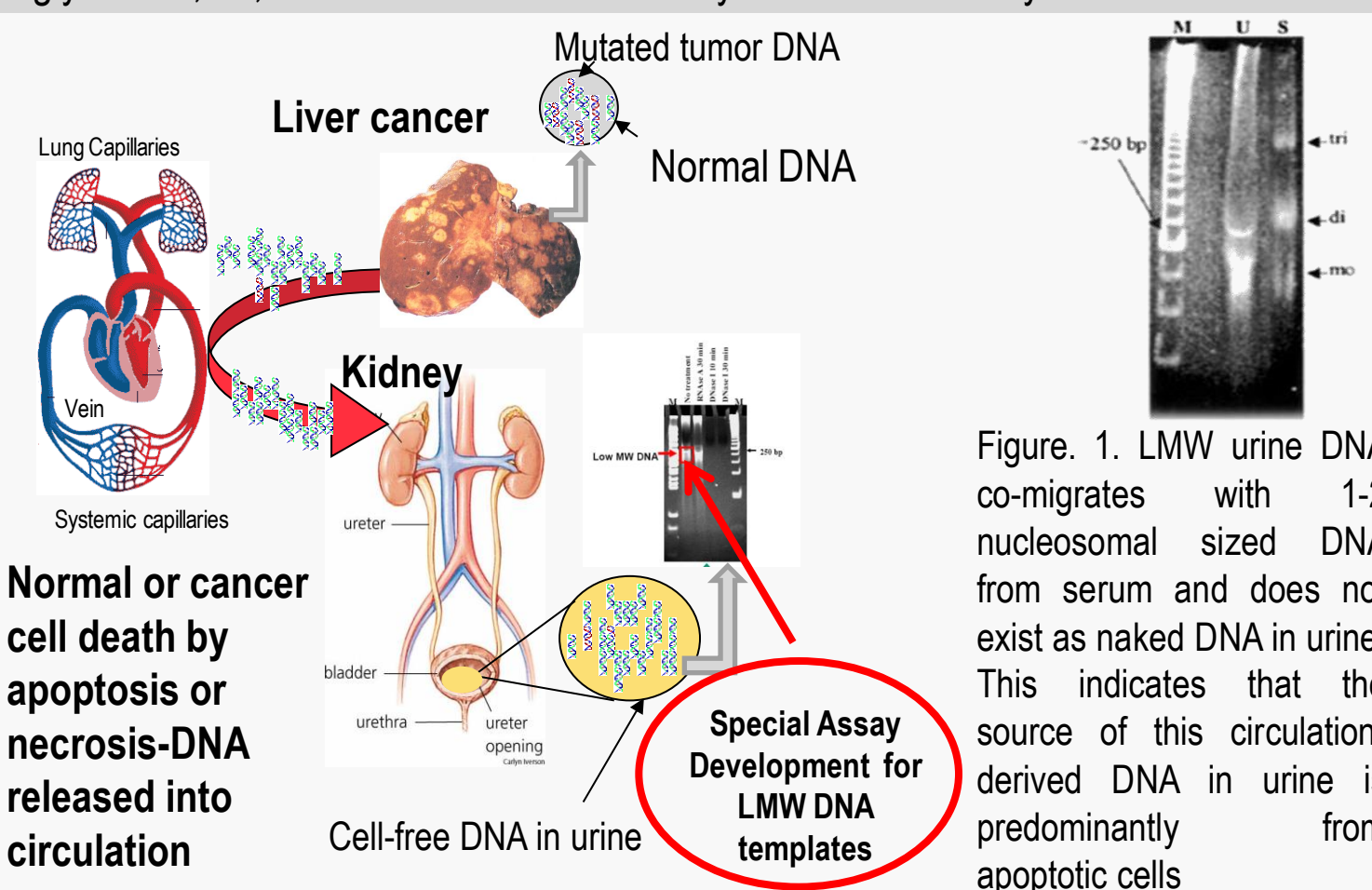


Figure 1. LMW urine DNA co-migrates with 1-2 nucleosomal sized DNA from serum and does not exist as naked DNA in urine. This indicates that the source of this circulation-derived DNA in urine is predominantly from apoptotic cells.

Urine contains fragmented, cell-free, cancer associated DNA, both mutated and methylated, derived from the circulation of cancer patients (Lin, Dhillion et al. 2011, Song, Jain et al. 2012, Jain, Xie et al. 2015) and the concentration of tumor-derived DNA in plasma and in urine is similar in patients with tumors (Su, Wang et al. 2008).

Table 1. Frequent genetic mutations (G) and epigenetic aberrant DNA methylations (M) reported in HCC tissues.

Pathway	Gene	DNA Alteration	Incidence (%)	Reported in circulation (Yes/No)
Wnt signalling	APC	M/G	80-93/ 1-2	Yes
	SFRP1	M	42-63	Yes
	CTNNB1	G	19-33	No
	AXIN1	G	13-15	No
Cell cycle/ Apoptosis	RASSF1A	M	55-85	Yes
	CDKN2A	M/G	50-70/ 5-10	Yes
	TP53	G	20-50	Yes
	IRF2	G	5	No
Detoxification	GSTP1	M	38-80	Yes
Telomerase maintenance	TERT	G	59	No

Methods

Urine samples were collected with written informed consent and institutional review board approvals from National Cheng Kung University (Tainan, Taiwan), Tzu-Chi Hospital, (Hualien, Taiwan), and Thomas Jefferson University Hospital (Philadelphia, PA), at visits with a hepatologist. Note the samples from TJU were barcoded for disease status as a blinded study. Total urine DNA was isolated (Su, Wang et al. 2004) and the low molecular weight urine DNA, DNA less than 1 kb, was obtained from total urine DNA using carboxylated magnetic beads (Agencourt Bioscience Corporation) as previously developed by us (Su, Wang et al. 2008). Bisulfite (BS) treatment was performed using EZ DNA Methylation-Lightning™ Kit (Zymo Research) following manufacturer's guidelines. In order to detect circulation-derived, cell-free DNA markers in urine, we have developed short amplicon (~50 bp) PCR-based assays for mutations in *TP53* (249T) (Lin, Dhillion et al. 2011), *CTNNB1* (exon 3 - codons 32-37), *TERT* (-124 promoter) and for aberrant DNA methylation in *GSTP1* (*mGSTP1*) and *RASSF1A* (*mRASSF1A*) (Jain, Xie et al. 2015).

Objective

Develop a urine test using a panel of select genetic and epigenetic markers for the early detection of primary and recurrent HCC.

Results

Case 1: Detection of a primary HCC

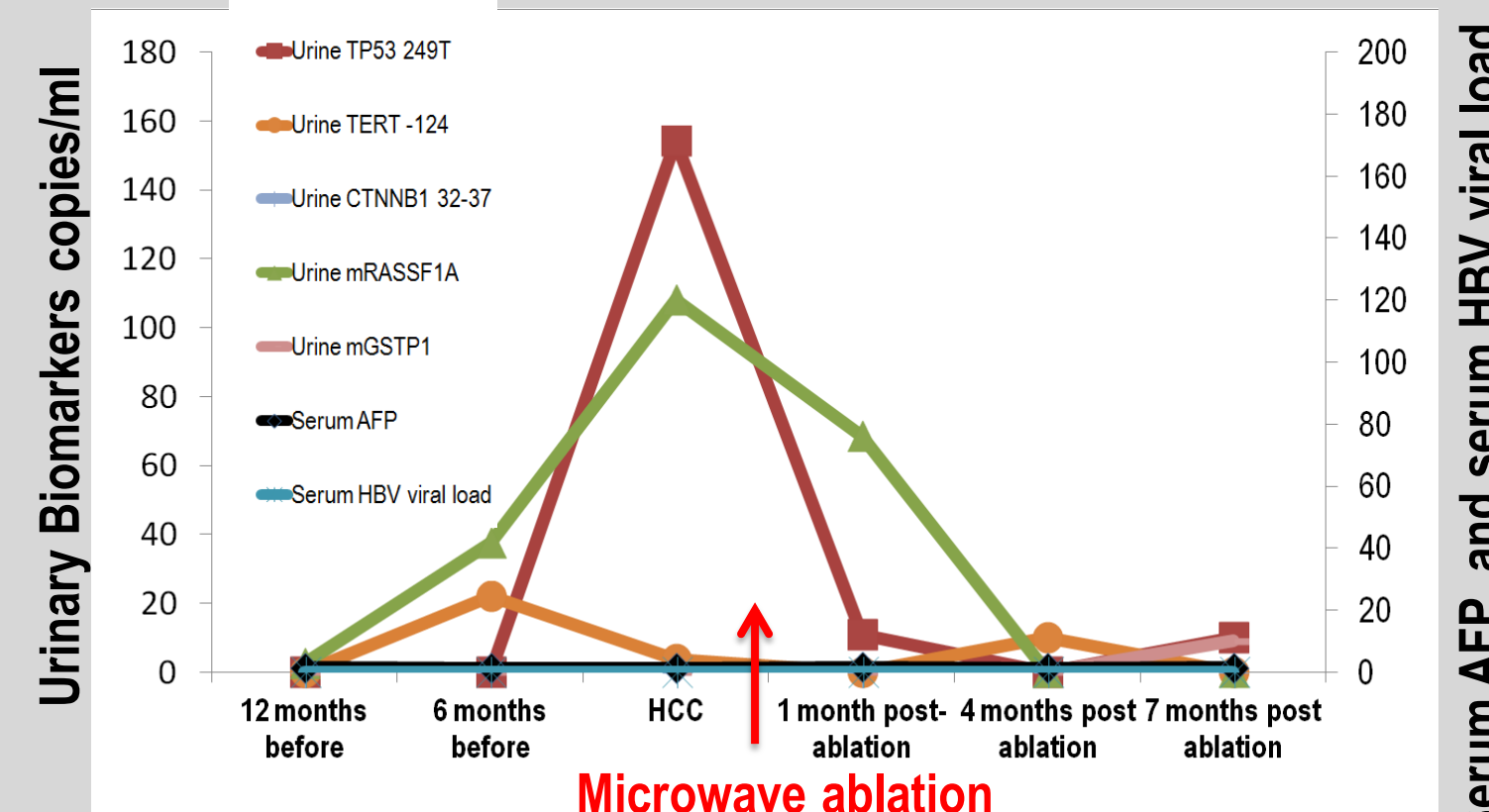


Figure 2: Retrospective analysis of DNA biomarkers from urine samples, plus serum AFP and serum HBV viral load levels collected during doctor visits of a 74 year-old male with HBV-cirrhosis who developed HCC that was undetectable by serum AFP and was treated with microwave ablation. Urinary *mRASSF1A* DNA and *TERT* 124 mutated DNA levels started to elevate 6 months prior to HCC detection by MRI and declined post treatment. *TP53* 249T mutation level rose with the appearance of HCC and declined post treatment.

Case 2: Detection of recurrent HCC

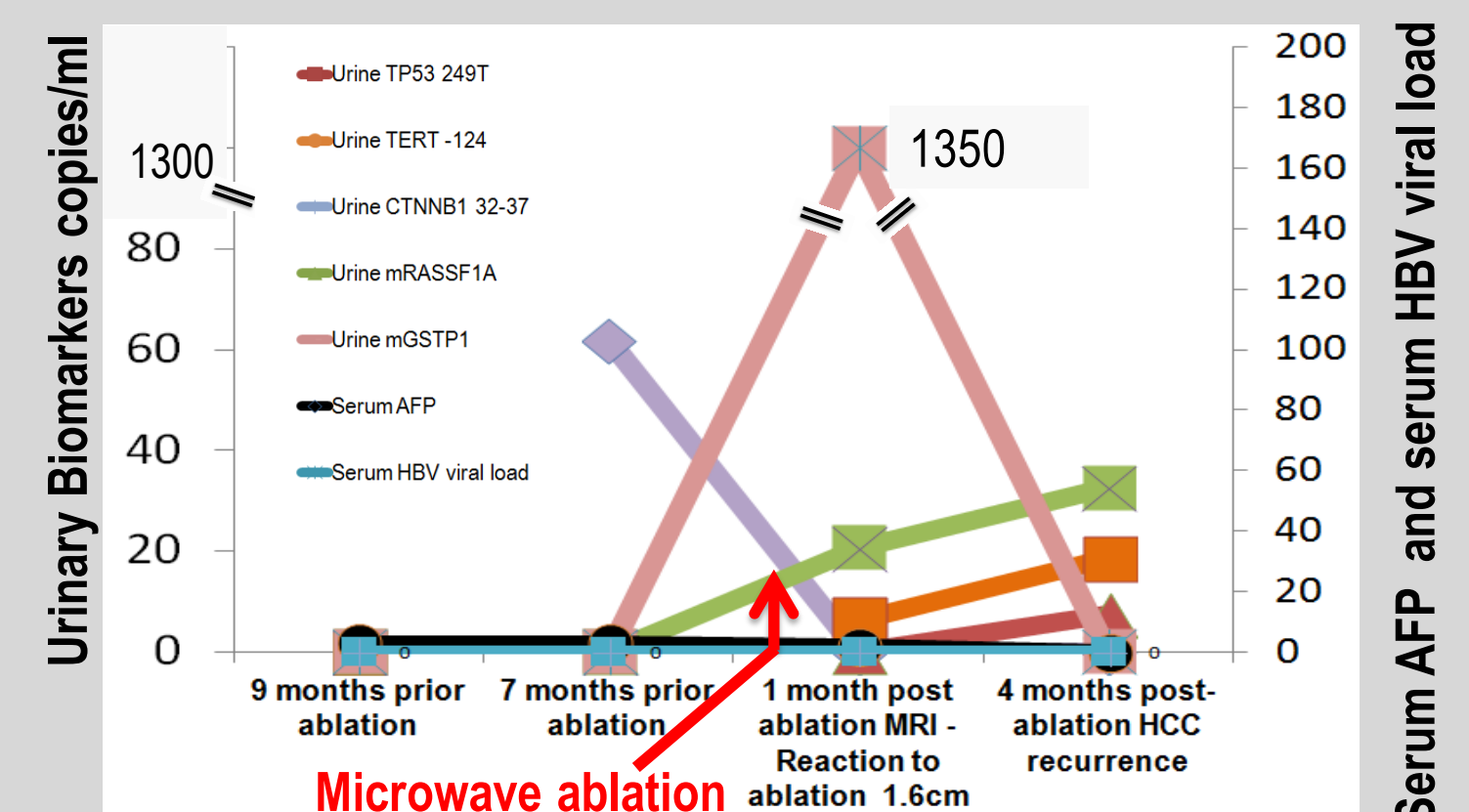


Figure 3: Retrospective analysis of urinary DNA markers plus serum AFP collected during doctor visits of a 55 year old male with HBV-cirrhosis who developed HCC that was undetectable by serum AFP but elevated urine *CTNNB1* mutation levels and was treated with microwave ablation. The patient, however, had a recurrence of HCC 4 months post treatment. While *mGSTP1* and *CTNNB1* mutation levels in urine declined post-treatment, urinary *mRASSF1A*, *TERT* 124 mutation and *TP53* 249T mutation levels continue to rise concurrent with the recurrence.

Results

Case 3: Detection of recurrent HCC

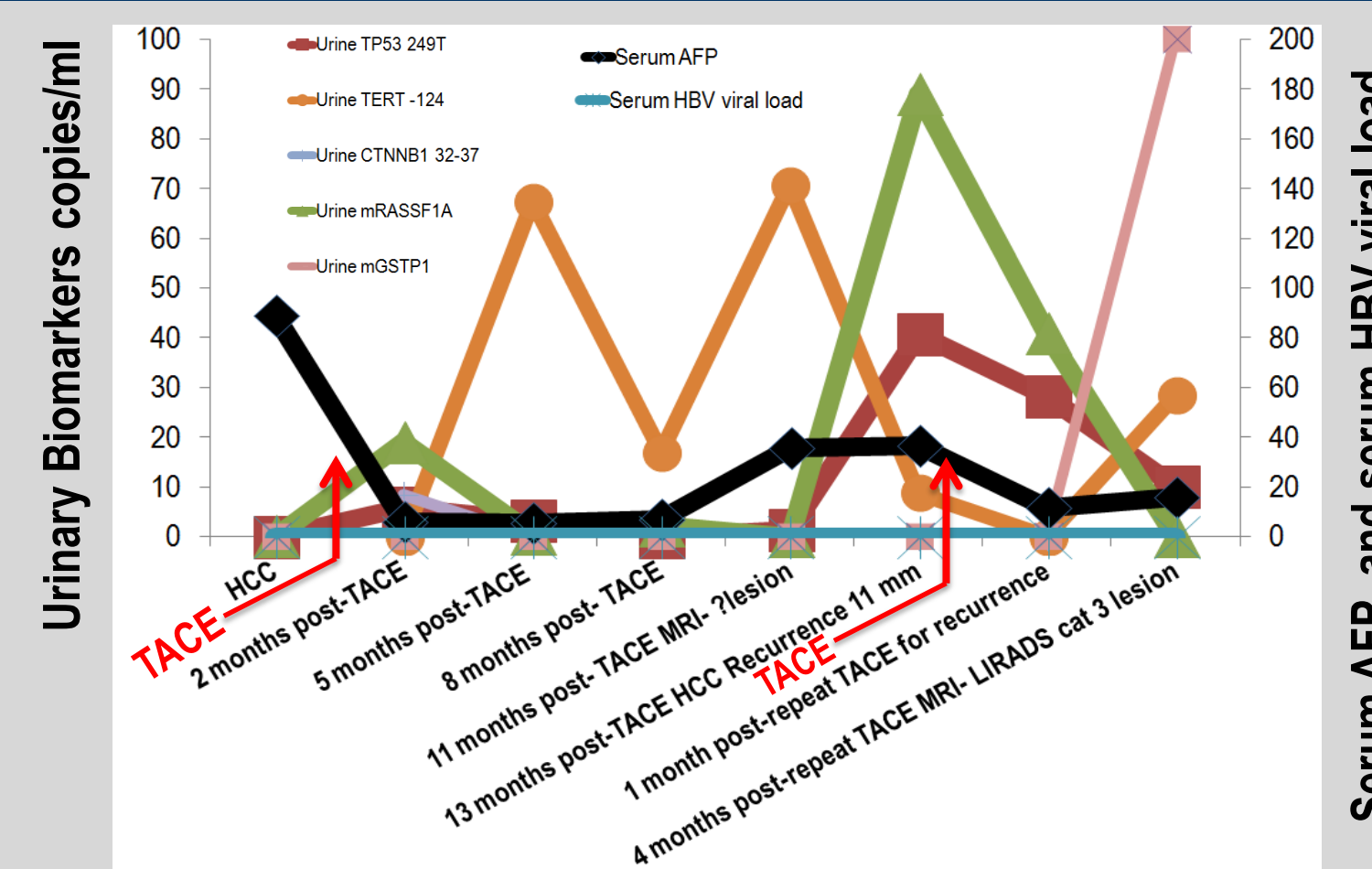


Figure 4: Retrospective analysis of urinary DNA biomarkers and serum AFP levels collected during doctor visits of a 73 year old male with HBV-cirrhosis and HCC that was treated with TACE. Samples were collected before TACE at the first time point. The DNA markers were undetectable but serum AFP was slightly elevated at 89 ng/ml and declined to baseline after TACE therapy. HCC recurrence was detected by MRI 11-13 months after TACE treatment. *TERT* 124 mutation levels are elevated 6 months prior to recurrence detection by MRI. *mRASSF1A*, *TP53* 249T mutation, and serum AFP levels rise with the appearance of recurrent nodule and declined post treatment. This recurrence was also treated with TACE. Note, MRI at 11 months was not definitive. Urine *mGSTP1*, *TERT* 124 levels rise 4 months post repeat TACE concurrent with the appearance of a LIRADS category 3 lesion on MRI.

Case 4: Detection of recurrent HCC

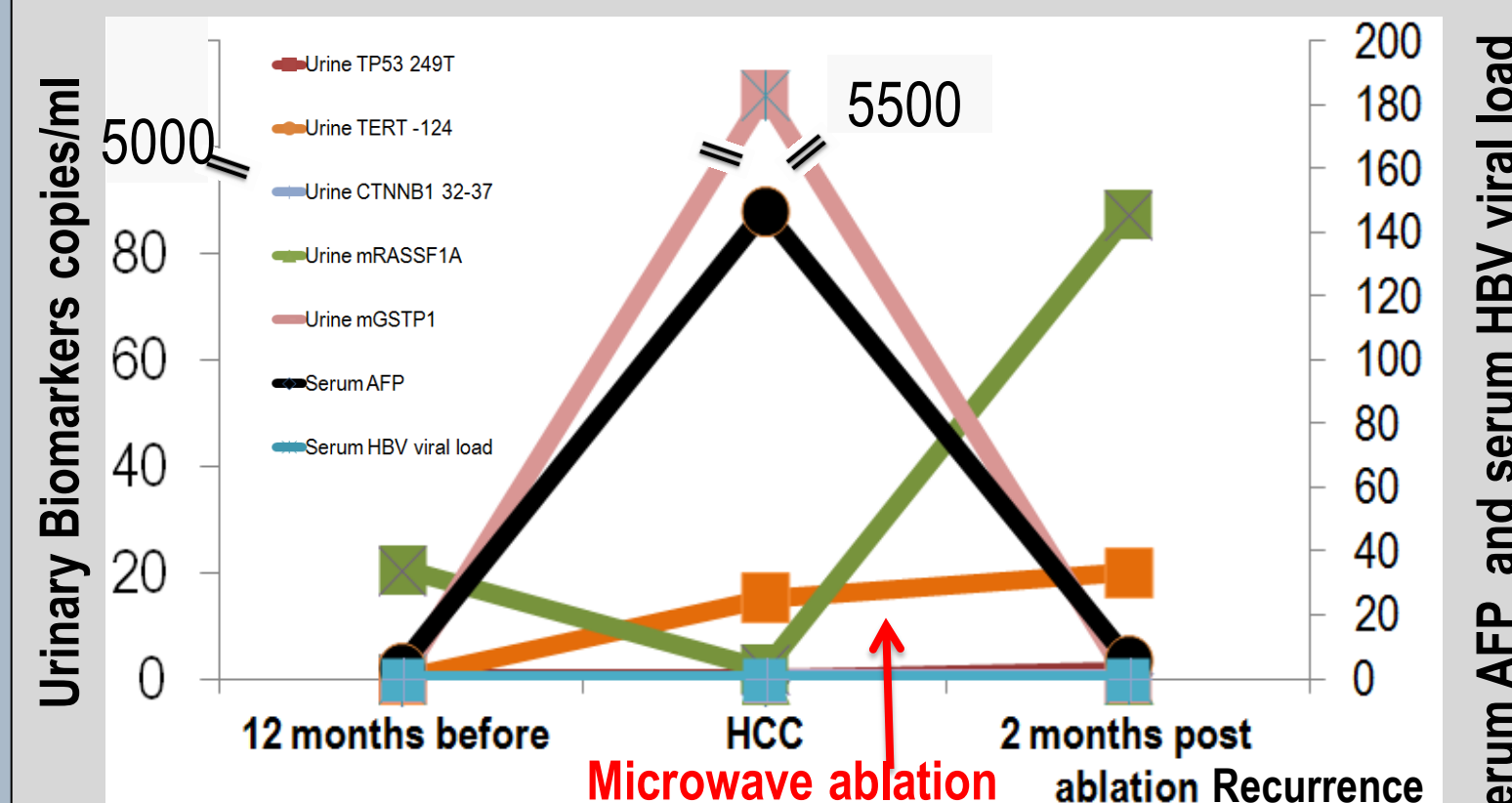
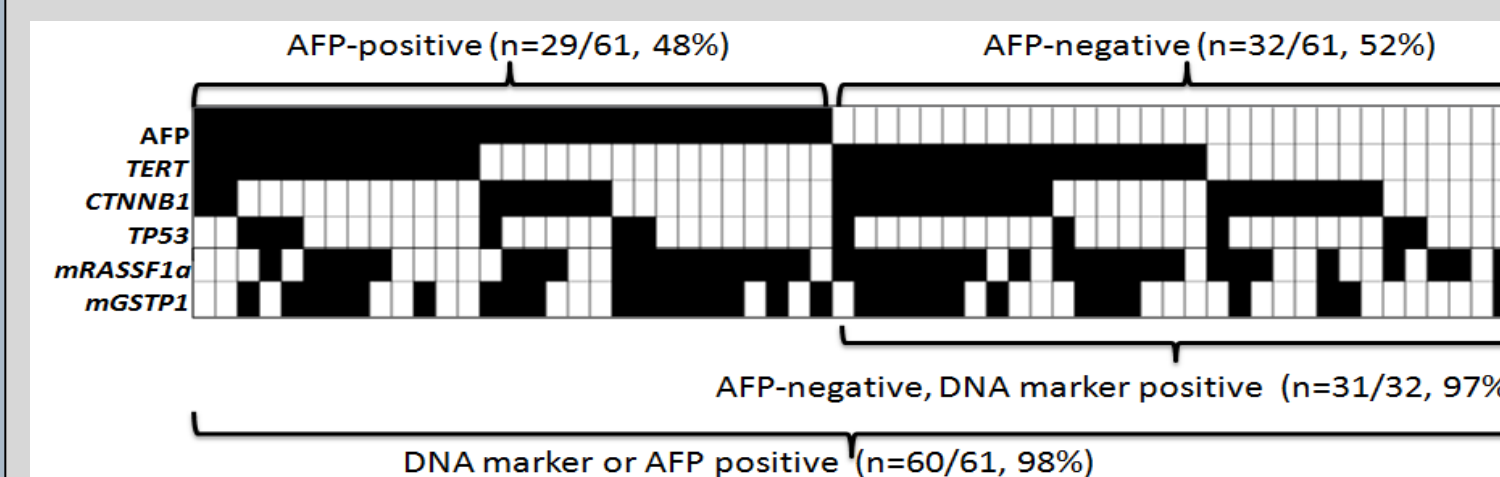


Figure 5: Retrospective analysis of urinary DNA markers plus serum AFP collected during doctor visits of a 53 year old male with HBV-hepatitis/early cirrhosis who developed HCC that was detectable by serum AFP and was treated with microwave ablation. The patient, however, had a recurrence of HCC 2 months post MW ablation. While *mGSTP1* level in urine declined post treatment, urinary *mRASSF1A* and *TERT* 124 mutation levels continued to rise concurrent with the recurrence.

Detection of AFP-negative HCC

(A) HCC tissue biomarker distribution



(B) HCC urine biomarker distribution

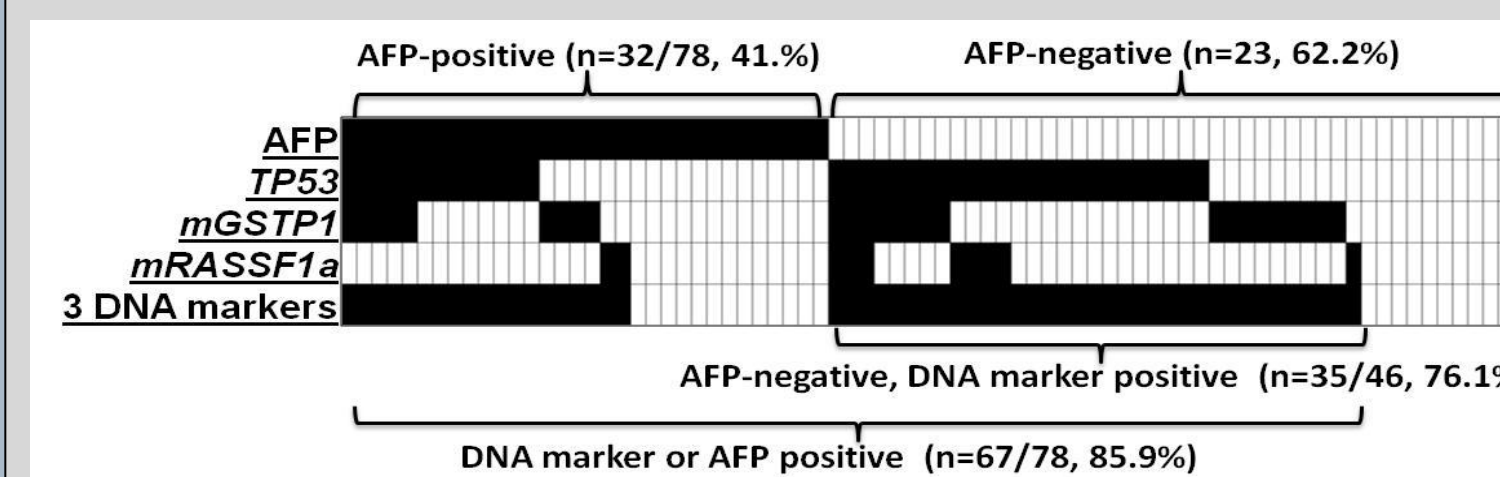


Figure 6. Detection of AFP-negative HCC by 5 HCC DNA markers in (A) tissue and (B) 3 cfDNA markers in urine. Marker distribution in individual HCC with available AFP value in our study population. Filled boxes represent a marker detected "positive", higher than cut-off value at 90% specificity distinguishing HCC from cirrhosis or positive for AFP. Empty boxes represent a marker value below the cut-off at 90% specificity or negative for AFP.

Conclusion

- A total of 10 cases with treated HCC were monitored by serum AFP and urine DNA biomarkers in a blinded study. Of the 10, 4 developed recurrence during the study. HCC-specific urine DNA markers were detected in 3 of 4 patients six months prior to MRI diagnosis and in one patient concurrent with MRI diagnosis.
- Fifteen cirrhosis and nine hepatitis patients were monitored every six months for HCC. In this group, one patient developed primary HCC and the urine DNA biomarkers were detected six months prior to MRI diagnosis in a blinded study.
- The urine test could lead to a paradigm shift for screening and effective management of primary and recurrent HCC and for personalized disease management.

Acknowledgements and Disclosures

This work is supported by R44 CA165312-02. Wei Song is the President and founder of JBS Science, Inc. Surbhi Jain is an employee of JBS Science, Inc.

